

CLAIMS

1. ~~A vector construct for the expression of streptavidin fusion proteins, comprising:~~

5 (a) a first nucleic acid sequence encoding genomic streptavidin or a functional variant thereof;

(b) a promoter operatively linked to the first nucleic acid sequence; and

(c) a cloning site for insertion of a second nucleic acid sequence encoding a polypeptide to be fused with streptavidin, interposed between the promoter and the first nucleic acid sequence.

10 2. The construct of claim 1, wherein said construct further comprises said second nucleic acid sequence inserted at said cloning site.

3. The construct of claim 1, wherein the promoter is an inducible promoter.

4. The construct of claim 3, wherein the promoter is the Lac promoter.

5. The construct of claim 1, wherein the promoter is a constitutive promoter.

15 6. The construct of claim 1, further comprising *S. avidinii* regulatory sequences interposed between the promoter and the cloning site.

7. The construct of claim 6, wherein the regulatory sequence is a streptavidin regulatory sequence.

20 8. The construct of claim 1, further comprising a bacterial leader sequence interposed between the regulatory sequences and the cloning site.

9. The construct of claim 8, wherein the leader sequence comprises a signal sequence.

10. The construct of claim 8, wherein the leader sequence comprises a *S. avidinii* streptavidin signal sequence.

5 11. The construct of claim 10, wherein the signal sequence comprises nucleotides 50 to 121 of Figure 4.

12. The construct of claim 1, further comprising a nucleic acid sequence that encodes a protein that is a selectable marker.

10 13. The construct of claim 12, wherein the protein confers antibiotic resistance.

14. The construct of claim 1, wherein the first nucleic acid sequence encodes at least amino acids 14 to 150 of streptavidin, Figure 4.

15. The construct of claim 1, wherein the first nucleic acid sequence encodes at least amino acids selected from the group consisting of 1 to 158, 5 to 158, 14 to 150, 14 to 151, 14 to 152, 14 to 153, 14 to 154, 14 to 155, 14 to 156, 14 to 157, or 14 to 158 of streptavidin, Figure 4.

16. A host cell transfected with the construct of claim 1.

17. The host cell of claim 16, wherein the cell is selected from the group consisting of a bacterium, an insect, a plant, and a mammalian cell.

Seed B3

18. A fusion protein, comprising at least a first and a second polypeptide joined end to end, wherein said first polypeptide comprises at least 129 amino acids of streptavidin, Figure 4, or functional variants thereof, and wherein said second polypeptide comprises an amino acid sequence differing by at least one residue from said first polypeptide.

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19. The fusion protein of claim 18, wherein said first and second polypeptides are separated by a linker of at least two amino acids.

Linker

20. The fusion protein of claim 19, wherein the linker is at least four amino acids.

Linker

21. The fusion protein of claim 20, wherein the linker is between four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, fifteen, sixteen, seventeen, eighteen, nineteen, and twenty amino acids.

22. The fusion protein of claim 21, wherein the linker is between five to ten amino acids.

15 23. The fusion protein of claim 18, wherein said second polypeptide is an antibody or a fragment thereof.

20 24. The fusion protein of claim 23, wherein said fusion protein is capable of forming a tetrameric complex with a second, third, and fourth fusion protein, said second, third, and fourth fusion protein comprising at least a first and a second polypeptide joined end to end, wherein said first polypeptide comprises at least 129 amino acids of streptavidin, Figure 4, or functional variants thereof, and wherein said second polypeptide comprises an amino acid sequence differing by at least one residue from said first polypeptide.

25. The fusion protein of claim 23, wherein the antibody is B9E9.

Sub B
26. The fusion protein of claim 23, wherein the antibody is a single-chain Fv
fragment (scFv).

27. The fusion protein of claim 26, wherein the single-chain Fv fragment is
5 derived from antibody B9E9.

28. The fusion protein of claim 26, wherein a linker connects the variable light
and variable heavy chains of the single chain antibody.

29. The fusion protein of claim 28, wherein the linker comprises at least ten
amino acid residues.

10 30. The fusion protein of claim 29, wherein the linker comprises at least
fifteen amino acids.

31. The fusion protein of claim 30, wherein the linker comprises at least
twenty amino acids.

Sub B
15 32. The fusion protein of claim 31, wherein the linker comprises at least four
Gly₄Ser linkers.

33. The fusion protein of claim 23, wherein the antibody is specific for a cell
surface protein or a cell-associated stromal or matrix protein.

34. The fusion protein of claim 33, wherein the antibody is a primatized
antibody.

35. The fusion protein of claim 33, wherein the antibody is a murine antibody.

36. The fusion protein of claim 33, wherein the cell surface protein or the cell-associated stromal or matrix protein is selected from the group consisting of CD20, CD45, EGP40, CEA, TAG72, NCAM, β -HCG, a mucin, and neoangiogenic antigens.

5 37. The fusion protein of claim 36, wherein the cell surface protein is CD20.

Sub B 38. The fusion protein of claim 18, wherein said first polypeptide comprises at least amino acids 14 to 150 of streptavidin, Figure 4.

10 39. The fusion protein of claim 18, wherein the first polypeptide comprises at least amino acids selected from the group consisting of 1 to 158, 5 to 158, 14 to 150, 14 to 151, 14 to 152, 14 to 153, 14 to 154, 14 to 155, 14 to 156, 14 to 157, or 14 to 158 of streptavidin, Figure 4.

15 40. A method for targeting a tumor cell comprising the administration of a fusion protein, said fusion protein comprising at least a first and a second polypeptide joined end to end, wherein said first polypeptide comprises at least 129 amino acids of streptavidin Figure 4, or conservatively substituted variants thereof, wherein said second polypeptide is a polypeptide which binds a cell surface protein, or a cell-associated stromal or matrix protein, on a tumor cell, wherein the fusion protein binds the cell surface protein, or a cell-associated stromal or matrix protein, on a tumor cell and wherein the streptavidin portion of the fusion protein is capable of binding biotin.

20 41. The method of claim 40, wherein the fusion protein binds a cell surface protein receptor, or a cell-associated stromal or matrix protein, on a tumor cell and a biotinylated radionuclide containing compound.

42. The method of claim 40, wherein said first and second polypeptides are separated by a linker of at least two amino acids.

43. The method of claim 42, wherein the linker is at least four amino acids.

44. The method of claim 43, wherein the linker is between four, five, six, 5 seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, fifteen, sixteen, seventeen, eighteen, nineteen, and twenty amino acids.

45. The method of claim 44, wherein the linker is five to ten amino acids.

46. The method of claim 40, wherein the second polypeptide is an antibody.

47. The method of claim 46, wherein the antibody is B9E9.

10 48. The method of claim 40, wherein the antibody is a single-chain Fv fragment (scFv).

49. The method of claim 48, wherein the single-chain Fv fragment is derived from antibody B9E9.

15 50. The method of claim 48, wherein a linker connects the variable light and variable heavy chains of the single-chain antibody.

51. The method of claim 50, wherein the linker comprises at least ten amino acid residues.

52. The method of claim 51, wherein the linker comprises at least fifteen amino acids.

53. The method of claim 52, wherein the linker comprises at least twenty amino acids.

5 54. The method of claim 53, wherein the linker comprises at least four Gly₄Ser linkers.

10 55. The method of claim 40, wherein the antibody specifically binds to a cell surface protein, or a cell-associated stromal or matrix protein, selected from the group consisting of CD20, CD22, CD45, CD52, CD56, CD57, EGP40, CEA, TAG-72, NCAM, -HCG, amucin, EGF receptor, IL-2 receptor, her2/neu, Lewis y, GD2, GM2, tenascin, sialylated tenascin, somatostatin, activated tumor stromal antigen, and neoangiogenic antigens.

56. The method of claim 40, wherein the antibody is a primatized antibody.

57. The method of claim 40, wherein the antibody is a mouse antibody.

15 58. The method of claim 40, wherein said first polypeptide comprises at least amino acids 14 to 158 of streptavidin Figure 4.

59. The method of claim 58, wherein said first polypeptide comprises at least amino acids 5 to 158 of streptavidin, Figure 4.

60. The method of claim 59, wherein said first polypeptide comprises at least amino acids 1 to 158 of streptavidin, Figure 4.

61. The method of claim 40, wherein the tumor cell is associated with a cancer selected from the group consisting of adenocarcinomas and hematological malignancies.

62. The method of claim 61, wherein the adenocarcinoma is selected from the group consisting of gliomas, prostate, ovarian, ~~breast, colon, rectal, pancreatic, and lung~~.

5 63. The method of claim 61, wherein the hematological malignancy is selected from the group consisting of non-Hodgkin's lymphoma, Hodgkin's lymphoma, T-cell lymphoma, acute lymphocytic leukemia, acute myelogenous leukemia, chronic lymphocytic leukemia, chronic myelogenous leukemia, multiple myeloma, and Waldenstrom's macroglobulinemia.

10 64. A method for constructing a tetravalent antibody, comprising contacting the fusion protein of claim 23, wherein said fusion protein is capable of forming a tetrameric complex, with a second, third, and fourth fusion protein, said second, third, and fourth fusion protein also capable of forming a tetrameric complex and comprising at least a first and a second polypeptide joined end to end, wherein said first polypeptide comprises at least 129 amino acids of streptavidin, Figure 4, or functional variants thereof, and wherein said second polypeptide comprises an amino acid sequence differing by at least one residue from said first polypeptide.

15 (a) b 65. A pharmaceutical composition, comprising the fusion protein of any one of claims 18-39.

66. A vector construct for the expression of streptavidin fusion proteins, comprising:
20 (a) a first nucleic acid sequence encoding a polypeptide to be fused with streptavidin;
(b) a promoter operatively linked to the first nucleic acid sequence; and

(c) a cloning site for insertion of a second nucleic acid sequence encoding at least one amino acids of streptavidin, Figure 4 or a functional variant thereof.

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